"Prevalence of Candida Species Causing Blood Stream Infection in Critically Ill Patients in Kanpur Up"

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Abstract:

Introduction: BSI by Candida, also known as candidemia, is defined as the isolation of Candida species from at least one blood culture in patients with symptoms or signs of a systemic infection. Candidemia was defined as the isolation of Candida in blood culture accompanied by fever, chills or hypotension and other corresponding clinical symptoms and signs and the exclusion of specimen contamination. The incidence of candidemia expressed as cases per 100,000 inhabitants has been reported to range from 1-8 cases and the prevalence was found to be 6.9 per 1000 cases according to a survey of Intensive Care Units (ICUs) worldwide.

Aim: To aim the identify the prevalence of Candida species causing BSI, in kanpur UP.

Material and Methods: This study was being conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur. A total of 100 specimens were inoculated into the blood culture bottles and incubated at 37°C for a maximum period of seven days. If positive then they blood was subcultured on cystene lactose electrolyte defieciency agar (CLED) incubated at 37°C. Gram staining was performed from the colony and the morphology of yeast cells was noted. Germ tube test was performed from colonies for presumptive identification of C. albican. Colonies from CLED were plated onto CHROM agar that is claimed to facilitate the isolation by colorimetric presumptive identification and were incubated at 37°C for 48 hours.

Results: A total of 100 blood samples, isolates of Candida species were obtained from 07(7%) positive blood culture cases as per inclusion criteria during the study period. Candida albicans (4/100, 4%), Candida parapsilosis (1/100, 1%) was the predominant species causing candidemia followed by Candida tropicalis (1/100, 1%), C. dubliniensis (1/100,1%), Candida glabrata 1/100, 1%), Candida auris and others species (00/100, 00%),

Conclusion: Even though CHROM agar helps with identification at a lower cost as compared to others automated methods, which is useful in countries having low resources.

Keywords: Candida albicans, Candida parapsilosis, Candidemia, CLED agar CHROM agar,

Introduction

In critically ill patients, bloodstream infection (BSI) is an important cause of morbidity and mortality. Candidemia is a leading cause of healthcare-associated BSI, with all cause in-hospital mortality reaching above 30 %.[1]. BSI by Candida, also known as candidemia, is defined as the isolation of Candida species from at least one blood culture in patients with symptoms or signs of a systemic infection [2]. Candidemia was defined as the isolation of Candida in blood culture accompanied by fever, chills or hypotension and other corresponding clinical symptoms and signs and the exclusion of specimen contamination [3]. The incidence of candidemia expressed as cases per 100,000 inhabitants has been reported to range from 1-8 cases [4] and the prevalence was found to be 6.9 per 1000 cases according to a survey of Intensive Care Units (ICUs) worldwide [5]. Risk factors associated with candidemia are premature birth, advanced age, long term hospitalization, prolonged exposure to antimicrobial drugs, invasive procedures such as using intravascular catheters, multiple interventions and the immunosuppressive conditions like diabetes, HIV and malignancy [6]. If the identified Candida species was C. albicans, CA-BSI was considered. Mixed-CA/B-BSIs were defined as the isolation of a bacterial organism from blood cultures obtained within 48 h before or after the onset of CA-BSI [7]. Although Candida albicans continues to be the predominant species to cause candidemia, recent studies have detected a growing proportion of BSI by NAC species like C. tropicalis, C. parapsilosis, C. glabrata, and C. krusei [8]. There has been a recent epidemiologic shift in candidemia driven by C.auris, a novel Candida spp., which is a multidrug-resistant pathogen causing protracted healthcare-associated outbreaks [9].

Timely management of candidemia is crucial to improve clinical outcome, as inadequate and delayed treatment initiation have been associated with increased mortality (10, 11). On these grounds, empirical antifungal therapy with echinocandins, fluconazole, or a lipid formulation of

amphotericin B (12) is often prescribed to patients with common predisposing factors for developing the infection, long before definitive identification and

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susceptibility testing results become available based on local epidemiological data. The epidemiology of candidemias may change over time and can vary significantly across different geographic regions and hospitals, depending on local factors (13, 14). Since empirical therapy relies on local epidemiology, continuous regional monitoring of temporal trends in incidence rate, species distribution, and susceptibility profiles of candidemic clinical isolates seems prudent. Finally, the socioeconomic situation may influence the epidemiology of infectious diseases (15). It is now essential to do the species-level identification as antifungal agent can be selected by the clinicians for the better patient care [16, 17]. Continuous surveillance is needed to know the incidence, species distribution of Candida causing BSI. The present study was designed with an aim to identify the spectrum of Candida species causing BSI on HI- CHROM agar.

Material and Methods

Study Setting: This study was being conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur.
Samples from already known patient
Study Design: Prospective study.
Type Of Study: Observational study.
Study Period: This study will be conducted from 2021 to 2022.
Size of Sample: Total 100 Blood samples taken from critically ill patients were collected following all the aseptic precautions at Rama Medical College Hospital and Research Centre as the source of the sample for the study.
Inclusion Criteria: All the patients who were having clinically suspected to septicemia and Candida isolates obtained as a single pathogen from blood cultures were included in the study.
Excision Criteria: Patients other than those of ICU admitted and also those who were under antibiotics or antifungal even from ICU admission status were excluded from this study.
Ethical Consideration: Ethical clearance will be taken from the institutional ethical committee.

Sample Processing: A total of 100 specimens were inoculated into the blood culture bottles and incubated at 37°C for a maximum period of seven days for detection of aerobic growth in blood samples and if there was no growth, the result was read as negative. In a case of any turbidity present in blood culture bottles indicates positive then they blood was subcultured on cystene lactose electrolyte defieciency agar (CLED) incubated at 37°C. CLED agar plates were examined for tiny,white to cream coloured, smooth and pasty colonies after 24-48 hours of incubation.
Gram staining was performed from the colony and the morphology of yeast cells was noted. All the isolates were processed by conventional methods. Germ tube test was performed from colonies for presumptive identification of C. albicans [18].

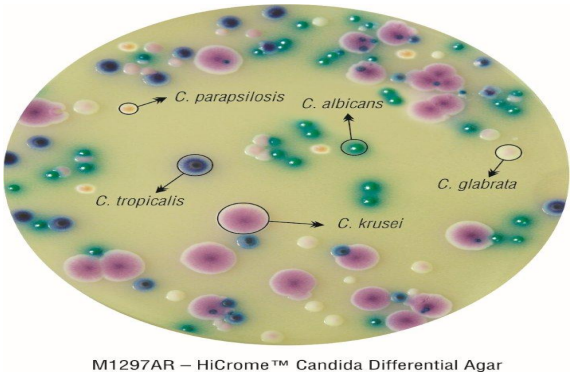
Colonies from CLED were plated onto CHROM agar (HiCrome Candida differential agar- Himedia), a differential culture medium that is claimed to facilitate the isolation by colorimetric presumptive identification [19] and were incubated at 37°C for 48 hours. The results were interpreted according to the colour of the colony formed and as per manufacturer's guidelines. All the isolates that gave doubtful morphology or not identified by conventional methods were taken as Candida spp.

Statistical Analysis: It was done as per Chi-square test to compare the conventional method.

Results

A total of 100 blood samples, isolates of Candida species were obtained from 07(7%) positive blood culture cases as per inclusion criteria during the study period. Male patients (65/100, 65%) predominated the female patients (35/100, 35%) in terms of isolation frequency. Maximum number of candidemia patients was in the 41-65 age group 50%, followed by 18-40 years 36% and patients were seen in the age groups of more than 65 years respectively. Overall most of cases had associated co-morbid conditions like malignancy, kidney disease, indwelling vascular catheters, surgical interventions and diabetes mellitus etc.
Candida albicans (4/100, 4%), Candida parapsilosis (1/100, 1%) was the predominant species causing candidemia followed by Candida tropicalis (1/100, 1%), C. dubliniensis (1/100,1%), Candida glabrata 1/100, 1%), Candida auris and others species (00/100, 00%),

C. albicans	Apple green
C. tropicalis	Metallic blue with a pink halo
C. dubliniensis	Dark green
C. glabrata	Pale pink to violet
C. parapsilosis	White to pale pink
C. krusei	Fuzzy pink coloured colonies



[Table/Fig-1]: Pigments produces of various Candida species on CHROMagar

[Table/Fig-2]: Socio-demographic and clinical profile of study subjects.

Variables	N - %
Gender	
Male	65 %
Female	35 %
Age (in years)	
18 to 40	36 %
41-65	50 %
≥65	14 %
Residence	
Urban	28 %
Rural	72 %
BSI associated with co - morbid conditions	
Malignancy	11%
Kidney disease	45%
Indwelling vascular catheters	34%
Surgical interventions	33%
Diabetes mellitus	55%

[Table/Fig-3]: Total Distribution of species of candida.

Species of Candida	Total (N)
C. albicans	4
C. tropicalis	1
C. dubliniensis	1
C. glabrata	0
C. parapsilosis	1
C. krusei	0
Others	0
Total	**07**

Discussion

S.No.	Study	Year	Results
1.	**Swati Jain[20]**	2020	This study was carried out in the Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, Odisha, India over a period of one and half year (January 2018-June 2019) the blood samples from 8156 ICU patients, clinically suspected to have septicaemia, were collected following all the aseptic precautions and before administration of antibiotics or antifungal
2.	**Nadeem Sajjad Raja[21]**	2020	This retrospective and prospective study was performed during January 2006 to June 2017. A total of 102 episodes of candidemia on 100 patients (55 males) were identified.
3.	**In the present study**	2022	This study was be conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur. The blood samples from 100 ICU patients, clinically suspected to have septicemia, were collected following all the aseptic precautions and before administration of antibiotics or antifungal

S.No.	Study	Year	Results
1.	**Swati Jain[20]**	2022	A total of 100 samples, male patients (55/100, 55%) predominated the female patients (45/100, 45%) in terms of isolation frequency. Maximum number of candidemia patients were in the elderly age group (>60 years, mean age- 71), i.e., 43%, followed by 41-60 years (mean age- 51.3) (33%); 14% and 10% patients were seen in the age groups of 21-40 years (mean age- 32) and up to 20 years (mean age- 8.4), respectively. Overall 60% of cases had associated co-morbid conditions like malignancy (29/100), kidney disease (40/100), indwelling vascular catheters (47/100), surgical interventions (26/100) and diabetes mellitus (52/100), etc. *Candida parapsilosis* (1/100, 1%) was the predominant species causing candidemia followed by *Candida tropicalis* (1/100, 1%), *Candida albicans* (4/100, 4%), *C. dubliniensis (1/100,1%)Candida auris* (0/100, 0%), *Candida glabrata* (0/100, 0%) and others (00/100, 0%)
2.	**Maria Siopi,et al[22]**	2019	The most commonly isolated *Candida* species was *C. albicans*, with a frequency ranging from 12.5% to 89.5%. Among NAC species, *Candida parapsilosis* species complex (SC) was the most frequently isolated (8.3 to 50%), followed by *Candida glabrata* SC (2 to 16%) and *Candida tropicalis* (3.3 to 21.4%)
3.	**In the present study**	2022	A total of 100 samples, male patients (65/100, 55%) predominated the female patients (35/100, 45%) in terms of isolation frequency. Maximum number of candidemia patients were in the elderly age group 41 -65 (50%), followed by 18 – 40 (36%); 14% and more than 65 years, respectively. Overall most of cases had associated co-morbid conditions like malignancy (11/100), kidney disease (45/100), indwelling vascular catheters (34/100), surgical interventions (33/100) and diabetes mellitus (55/100), etc. *Candida parapsilosis* (26/100, 26%) was the predominant species causing candidemia followed by *Candida tropicalis* (23/100, 23%), *Candida albicans* (21/100, 21%), *Candida auris* (15/100, 15%), *Candida glabrata* (5/100, 5%) and others (10/100, 10%)

S. No.	Study	Year	Results
1.	**Bhattacharjee P[23]**	2016	A total blood samples, isolates of *Candida* species were obtained from 4.03% positive blood culture cases as per inclusion criteria during the study period.
2.	**Gandham NR et al.[24]**	2016	A total of blood samples, isolates of *Candida* species were obtained from 14.08%) positive blood culture cases as per inclusion criteria during the study period.
3.	**In the present study**	2022	A total of 100 blood samples, isolates of *Candida* species were obtained from 07(7%) positive blood culture cases as per inclusion criteria during the study period.

Results

The diagnosis was recorded and the specimen was collected by attending ophthalmologist from all study participants presented with Ocular and per ocular infections. In our study, Out of the total 250 samples taken in our study, participants with ocular and per ocular infections, 180 (72%) were culture positive. Among the culture positive isolates, the proportions of clinical finding were mainly conjunctivitis (70), dacryocystitis (40), blepharitis (40), and blephero-conjunctivitis (30) from the total study participants; the Males were 120 (66.7%) whereas Females were 60(33.5%). The maximum number of cases was in the age group above 40 years followed by 30-40 years of age and least was recorded in the age group of 10 years and below Years. The maximum number of cases were maximum in the Rural area with 130(72.2%) then in Urban area were 50 (27.7%). And the number of cases was more in rural areas in which the maximum number of cases were of the students and the farmers in our study.

In our study the number of Gram-positive and Gram-negative bacteria were 120 (66.6%) and 60 (33.3%) respectively. Among the growth Staphylococcus aurous

was the most common isolated in Gram-positive bacteria and in Gram-negative bacteria, Escherichia coli was the most predominant isolate.

Table no.1 Gender wise Distribution of bacteria isolated from the study

Gender	Number Of Isolates	Percentage
Male	120	66.7%
Female	60	33.5%

Table no.2 Distribution of bacteria isolated from study participants with ocular and per ocular infections based on their Gram reaction,

Bacteria based on Gram reaction	Isolated bacteria	Number of isolates	(%)
Gram positive bacteria	*S. aureus*	65	54.10%
	CoNS	50	41.60%
	S. pneumoniae	5	4.10%
Gram negative bacteria	*E. coli*	20	33.30%
	Klebsiella pneumonia	17	28.30%
	Moraxella spp.	7	11.60%
	Citrobacter spp.	4	6.60%
	N. gonorrhoeae	3	5%
	H. influenzae	3	5%
	N. meningitidis	2	3.30%
	Pseudomonas spp.	2	3.30%
	Proteus mirabilis	2	3.30%
Total		180	

From our study the number of Gram-positive 111 (92.5%) was susceptible to gentamicin, clindamycin, and erythromycin respectively. Among the gram positive isolates S. aureus was the common isolate foillowed by Cons. In 65 S. aureus, 35(53.8%) and 30(46...1%) were resistant to penicillin and tetracycline respectively. and Gram-negative bacteria were 60 (33.3%) respectively. In which E.coli was the most dominant followed by Klebsiella pneumonia.

Among the growth Staphylococcus aureus was the most common isolated in Gram-positive bacteria and in Gram-negative bacteria, Escherichia coli was the most predominant isolate.

Majority of CoNS were resistant to penicillin and tetracycline All S. pneumonia isolates were susceptible to penicillin Among 60 Gram-negative bacteria isolated 50(83.3%) were susceptible to ciprofloxacin, and ceftriaxone respectively.

Discussion

In our study, a total of 250 patients seeking treatment for eye infection at Rama tertiary care centre were studied. Out of the total 250 samples taken in our study, 180 (72%) were culture positive. Among the culture positive isolates, the proportions of clinical finding were mainly conjunctivitis (70), dacryocystitis (40), blepharitis (40), and blephero-conjunctivitis (30) [7].

From the total study participants, the Males were 120 (66.7%) whereas Females were 60 (33.5%) which was similar to the study by Mshangila B [8] and Akililu [9]. In our study Gram positive bacteria was more than the Gram negative bacteria, which supported our study by other authors [9] [10]. In the current study, the predominant bacterial isolates were S. aureus (54.1%) followed by CoNS (41%) and then S. pneumonia (4.1%). The finding of this study is comparable with previous studies conducted in Ethiopia [11, 12, and 13] and India [14]. In the Gram negative bacteria, E.coli was the most common isolate which was supported by other study also [12, 15]. In this study, the majority of bacteria were resistant to tetracycline and penicillin, while most of the E.Coli isolates were susceptible to ciprofloxacin. This finding is in agreement with the study conducted in Gondar, Ethiopia [16]. The reason for increased resistance to penicillin and tetracycline may be prior exposure of the isolates to these antibiotics. Moreover, these antibiotics are common and patients can access them easily with low price. According to Getahun et al. [11] previous use of antimicrobials and duration of present illness was significantly associated with bacterial eye infection. Proper Screening programs specially to avoid bacterial colonization of the isolates should be check [17].

Limitation of the Study

Identification of the bacteria in this study does not necessarily mean that the isolated bacteria were responsible for the infection/inflammation.

Conclusion

Treatment of bacterial eye infections involves is very important with its empirical treatment with topical ophthalmic .Broad-spectrum antibiotic formulations that become a prevailing practice among ophthalmologists and general practitioners. This along with the irrational use of drugs, availability of antibiotics without prescription, has led to the development of resistance to commonly used antibiotics, therefore strict regulation policy should be followed to stop the misuse of the drug.

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